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Extraction of antioxidant pectic-polysaccharide from mangosteen (*Garcinia mangostana*) rind: Optimization using response surface methodology

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ABSTRACT

Box-Behnken design (BBD) was employed to optimize the incubator temperature (X_1: 50–80 °C), extraction time (X_2: 2–4 h) and pH (X_3: 2–4) to obtain a high antioxidant pectic-polysaccharide yield with high uronic acid content and antioxidant activity from mangosteen rind. Analysis of variance showed that the contribution of quadratic model was significant for the extraction yield and antioxidant activity whereas linear model was significant for pectin content. Optimization study using response surface methodology was performed and 3D response surfaces were plotted from the mathematical model. Two optimal conditions were given: condition (1) X_1 = 80.0 °C; X_2 = 3.93 h; X_3 = 2.45, and condition (2) X_1 = 67.7 °C; X_2 = 3.67 h; X_3 = 2.00. These optimum conditions yielded pectic-polysaccharide of ~12.0–12.4%, uronic acid content of ~20.2–21.1 mg/g, and %DPPHsc/g extract of 225–252, respectively. Close agreement between experimental and predicted values was found. This could therefore be applied in extraction of mangosteen-derived functional pectic-polysaccharide in industry.

1. Introduction

Lignocellulosic biomass is the most abundant plant-derived materials (e.g. agricultural residues, herbaceous crops, forestry wastes, woods) that have been left behind by the agro-food industries due to the modern agriculture practices that had increased food production yield tremendously and parallel to this is the amount of waste generated from this large scale cultivation. This occurrence has brought up a serious concern in environmental issues as well as the alternatives to solve the problems encountered. Globally, part of these waste materials have been used for animal feed and fertilizer (Mamma, Kourtoglou, & Christakopoulos, 2008), however, a large portion of these materials is still deposited annually. In recent decades, lignocellulosic biomass has attracted attention in biorefinery process. It was suggested that this material could be the largest potential feedstock for bioethanol production (Huang, Ramaswamy, Tschirner, & Ramarao, 2008; Kim et al., 2010; Kszos, 2006). Apart from bioethanol, natural products should also be considered. Therefore, food, nutraceutical and pharmaceutical industries have come into place where the industries are searching for new ingredients from natural sources (Guerrero, Torres, & Nuñez, 2008; Kasankala, Xue, Weilong, Hong, & He, 2007; Levigne, Ralet, & Thibault, 2002; Masmoudi et al., 2008; Wu, Cui, Tang, & Gu, 2007).

Apart from cellulose, all plant cell walls have a similar structure that consists of pectins (also known as pectic-polysaccharides) (Lerouxel, Cavalier, Liepman, & Keegstra, 2006). These plant-derived materials are widely used as gelling agents, thickeners, stabilisers, emulsifiers and fat-substitutes, and are listed as ingredients in numerous food products (Rolin, Nielsen, & Glahn, 1998; Willats, Knox, & Mikkelsen, 2006). Citrus peels and apple pomace are currently the important sources of pectin manufacturing, whilst other potentially valuable sources remain largely unexplored. Recent published work showed that *Parkia speciosa* pod could produce functional pectic-polysaccharide (Gan, Abdul Manaf, & Latiff, 2010a, 2010b). Wong, Alkarkhi, and Easa (in press, 2010) have also extracted this pectic-polysaccharides from durian rind and found that this extracted pectic-polysaccharide could act as biosorbent to remove heavy metal such as lead, nickel, and copper. Water-soluble pectin extracted from a durian rind was also found to have wound healing properties (Hokputsa et al., 2004). Other health effects of pectins, such as lowering cholesterol and serum glucose levels (Yamada, 1996; Behall & Reiser, 1986), inducing apoptosis in human colonic adenocarcinoma cells (Olano-Martin, Rimbach, Gibson, & Rastall, 2003) and anticancer activities (Yamada, Kiyohara, & Matsumoto, 2003), were also evident. Hence, this search for plant-derived biomaterials has therefore stimulated research interest in producing functional components from under-utilized bulk agro-waste, such as fruit peels.

In the current study, mangosteen (*Garcinia mangostana*) rind was used. Mangosteen, known as "queen of fruits", is cultivated in Southeastern Asia. The fruit is whitish colour with soft texture whereas the rind is firm and dark purple in colour. Traditionally,

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Table 1
Experimental domain of Box-Behnken design (BBD).

Variables	X_j		Factor levels		
	Uncoded	Coded	−1	0	1
Temperature (°C)	X_1	x_1	50	65	80
Time (h)	X_2	x_2	2	3	4
pH	X_3	x_3	2	3	4

the latter is used for skin infection and wound treatments. It is also widely used to against inflammation, diarrhea, cholera and dysentery. Other medicinal properties of the rind were listed by Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra, and Pérez-Rojas (2008). Up to date, several researches have demonstrated that mangosteen rind possessed antioxidant, antitumoral, antiallergy, antiinflammation, antibacterial and antiviral activities. In this matter, xanthones were claimed to be responsible for these biological activities (Suksamrarn et al., 2006; Pedraza-Chaverri et al., 2008). Preliminary study showed that the extract possessed high antioxidant pectic-polysaccharide. Following the discovery of medicinal phytochemicals, extraction of antioxidant pectic-polysaccharide from mangosteen rind should also be conducted. This material may suggest possible therapeutic applications that related to mangosteen in future.

The objectives of this study were to explore the potential of mangosteen rind in producing pectic-polysaccharide and to optimize the conditions for the extraction of pectic-polysaccharide that obtain high extraction yield, uronic acid content and antioxidant activity (i.e. %DPPHsc/g extract). Response surface methodology (RSM) was applied to fit and to exploit a mathematical model representing the relationship between the responses (i.e. extraction yield, uronic acid content and %DPPHsc/g extract) and variables (i.e. temperature, extraction time and pH).

2. Materials and methods

2.1. Materials

One batch of 20 kg of mangosteen was purchased from local market (Air Itam market) located in Penang, Malaysia. The raw samples were rinsed with distilled water to remove other impurities (such as dust and pesticide). The rinds were immediately separated from the fruits and the former was lyophilised and milled. The powder obtained was sieved (60-mesh size screen) and stored at 4 °C until use. All chemicals (ethanol, disodium phosphate and citric acid) used in the experiment were of analytical grade and were purchased from Sigma–Aldrich (Malaysia).

2.2. Extraction of pectic-polysaccharide

Extractions were carried out in a conical flask placed in an incubator shaker (IKA KS 4000i, Germany) as follows according to Masmoudi et al. (2008): 1.0 g of lyophilised mangosteen rind powder (MRP) was added to 50 ml of the citrate–phosphate buffer (solid–liquid ratio: 1:50, w/v) at different pH in each flask. The pH's of the mixtures were adjusted with 0.1 M HCl/NaOH and subsequently incubated in an incubator with constant agitation (250 rpm) at different incubation temperatures and times (Table 1). The resulting slurries were immediately filtered through a muslin cloth after incubation. The filtrates were then centrifuged at 20 °C for 30 min at 5000 × *g* to remove the remaining solid particles. Two volumes of 95% (v/v) ethanol were subsequently added to one volume of the extracts in order to precipitate the extracted pectic-polysaccharide (EPP). The obtained mixtures were kept for 2 h at 4 °C prior to filtration. The precipitates were washed three times with 50, 75 and 100% ethanol and filtered in order to remove the mono- and disaccharides. The EPP were then dried at 50 °C to a constant weight. The extraction yields (*Y*), subject of this study, were calculated as follows:

$$Y\ (\%,\ \text{w/w}) = \frac{W_{EPP}}{W_{MRP}} \times 100 \quad (1)$$

where W_{EPP} was defined as weight of EPP whereas W_{MRP} was defined as weight of MRP used.

2.3. Determination of uronic acid

Uronic acid was determined by m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Samples (0.5 ml) were mixed thoroughly with 3.0 ml of 0.0125 M sodium tetraborate solution (in concentrated sulphuric acid) in an ice bath. The mixtures were heated in a boiling bath for 5 min and subsequently cooled in an ice bath. The mixtures were added with 0.05 ml of 0.15% *m*-phenylphenol (in 0.5% sodium hydroxide solution). The absorbances at 520 nm were recorded after standing for 5 min. Standard curve was obtained using galacturonic acid (0–100 μg/ml).

2.4. Antioxidant activity

The DPPH free radical scavenging activity of each sample was determined according to Liu et al. (2009). EPP was reconstituted with distilled water and pre-diluted (20×). Aliquots of each sample (0.1 ml) were added to 3 ml of methanolic DPPH solutions (0.1 mM). Discolorations were measured at 517 nm after incubation for 30 min at 30 °C in the dark. The %DPPH which was scavenged ($\%DPPH_{sc}$) was calculated using:

$$\%DPPH_{sc}/\text{g extract} = \left\{ \frac{A_{cont} - A_{sample}}{A_{cont}} \right\} \times \frac{100}{W_{EPP}} \quad (2)$$

where A_{cont} was defined as absorbance of the control, A_{sample} was defined as absorbance of the sample (the extracts) whereas W_{EPP} was defined as weight of EPP.

2.5. Experimental design

The extraction parameters were optimized using RSM. A Box-Behnken design (BBD) was employed in this regard. Incubator temperature (X_1), extraction time (X_2) and pH (X_3) were chosen for independent variables. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments. The experimental design consists of twelve factorial points and five replicates of the central point (Table 2). Yields of EPP, uronic acid content and %DPPHsc/g extract were selected as the responses for the combination of the independent variables given in Table 2. Three experiments of each condition were carried out and the mean values were stated as observed responses. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the equation:

$$x = \frac{(X_i - X_o)}{\Delta X} \quad (3)$$

where *x* is the coded value, X_i is the corresponding actual value, X_o is the actual value in the center of the domain, and ΔX is the increment of X_i corresponding to a variation of 1 unit of *x*.

The mathematical model corresponding to the composite design is:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} \beta_{ij} X_i X_j + \varepsilon \quad (4)$$

Table 2
BBD with the observed responses and predicted values for yield of pectic-polysaccharide (%), uronic acid content (mg/g) and %DPPHsc/g extract.

Run	Coded variable levels			Observed (Y_1)[a]			Predicted (Y_o)		
	x_1 (temperature)	x_2 (time)	x_3 (pH)	Yield (%)	Uronic acid content (mg/g)	%DPPHsc/g extract	Yield (%)	Uronic acid content (mg/g)	%DPPHsc/g extract
1	1	0	1	23.5	9.4	189.3	22.5	10.9	189.8
2	−1	0	1	23.1	5.5	85.4	24.0	4.8	86.4
3	1	1	0	13.8	18.5	251.2	14.3	17.4	250.0
4	0	−1	1	23.2	6.3	136.4	22.7	7.3	135.1
5	1	−1	0	14.2	16.4	193.6	15.7	16.1	195.3
6	1	0	−1	12.7	20.0	229.5	11.7	22.6	228.5
7	−1	1	0	19.4	9.6	131.7	17.9	11.3	130.0
8	0	0	0	16.2	14.1	215.2	14.5	13.7	222.9
9	0	1	−1	12.1	20.6	208.9	12.6	20.2	212.0
10	0	0	0	14.2	18.3	224.4	14.5	13.7	222.9
11	0	0	0	13.9	14.2	215.3	14.5	13.7	222.9
12	−1	0	−1	15.4	15.6	155.8	16.4	16.5	155.3
13	0	1	1	21.1	7.2	158.3	21.7	8.5	158.1
14	0	0	0	14.5	14.2	227.4	14.5	13.7	222.9
15	0	−1	−1	14.1	18.8	190.5	13.5	18.9	188.9
16	0	0	0	13.9	15.2	232.2	14.5	13.7	222.9
17	−1	−1	0	18.8	9.4	137.5	18.3	10.0	138.7

[a] Mean of triplicate determination.

where Y is the dependent variables (extraction yield, uronic content or %DPPHsc/g extract), β_o is the model constant, β_i, β_{ii} and β_{ij} are the model coefficients and ε is the error. They represent the linear, quadratic and interaction effects of the variables. Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (version 6.0, USA). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

2.6. Statistical analysis

Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Duncan's test. Statistical analyses ($p < 0.05$) were performed using Statistical Package for Social Science for Window, version 12.0 (SPSS Institute Inc., Cary NC). The optimal extraction conditions were estimated through regression analysis and three-dimensional response surface plots of the independent variables and each dependent variable.

3. Result and discussion

Effects of extraction temperature (X_1), time (X_2) and pH (X_3) on extraction yield, uronic acid content and antioxidant activity were studied during experimentation. These parameters were chosen during the preliminary study which gave the highest yield of EPP and uronic acid content with desired antioxidant activity. The results of 17 runs using BBD are given in Table 2 that include the design, observed responses and the predicted values. Close agreement between experimental and predicted values was found. Result also showed that the yield of EPP ranged from 12.1 to 23.5%. The maximum yield (23.5%) was found under the experimental conditions of $X_1 = 80\,^{\circ}C$, $X_2 = 3.0$ h and $X_3 = 4.0$. A wide range of uronic content was also found (5.5-20.6 mg/g) and the maximum point (20.6 mg/g) was found in conditions of $X_1 = 65\,^{\circ}C$, $X_2 = 4$ h and $X_3 = 2.0$. On the other hand, the antioxidant property (%DPPHsc/g extract) ranged from 85.4 to 251.2%/g. The highest %DPPHsc/g extract (251.2%/g) value was found in conditions of $X_1 = 80\,^{\circ}C$, $X_2 = 4$ h and $X_3 = 3$. These conditions varied depending on the response required. Therefore, optimum process condition should be investigated in order to obtain high extraction yield, uronic acid content and antioxidant activity.

3.1. Model fitting

Table 3 presents the results of fitting models (quadratic or linear) to the data. The results of analysis of variance (ANOVA) indicate that the contribution of quadratic model was significant for responses of extraction yield and antioxidant activity whereas linear model was significant for response of uronic acid content. The fitted quadratic models for extraction yield and %DPPHsc/g extract in coded variables are given in Eqs. (5) and (7), respectively. On the other hand, Eq. (6) represents the fitted linear model for uronic acid content. The significance of each coefficient was determined using the F-test and p-value in Table 3. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller (Atkinson and Donev, 1992).

$$\text{Extraction yield} = 14.53 - 1.56x_1 - 0.48x_2 + 4.57x_3 + 1.52x_1^2 + 0.50x_2^2 + 2.62x_3^2 - 0.24x_1x_2 + 0.79x_1x_3 - 0.003x_2x_3 \quad (5)$$

$$\text{Uronic acid content} = 13.72 + 3.05x_1 + 0.63x_2 - 5.83x_3 \quad (6)$$

$$\%\frac{\text{DPPHsc}}{\text{g extract}} = 222.89 + 44.15x_1 + 11.51x_2 - 26.91x_3 - 26.47x_1^2 - 17.93x_2^2 - 31.43x_3^2 + 15.85x_1x_2 + 7.54x_1x_3 + 0.88x_2x_3 \quad (7)$$

3.1.1. Extraction yield

It can be observed that the variable with the largest effect on extraction yield was linear term of pH (X_3) followed by quadratic term of pH (X_3^2) and linear term of extraction temperature (X_1) (Table 3). However, linear term of extraction time (X_2), quadratic terms of extraction temperature and time (X_1^2 and X_2^2) and all the interaction terms (X_1X_2, X_1X_3 and X_2X_3) were found insignificant ($p > 0.05$). Results shown in Table 3 suggested that only the change of extraction temperature and pH had significant effects ($p < 0.05$) on the yield of EPP. Extraction time however did not give any significant ($p > 0.05$) contribution to the yield. This might due to the time required for the extraction has reached its maximum point after 2 h of incubation. The coefficient of determination (R^2) of the

Table 3
ANOVA for response surface models: estimated regression model of relationship between response variables (yield, uronic acid content and %DPPHsc/g) and independent variables (X_1,X_2,X_3).

Source	Sum of squares	DF	Mean square	*F*-value	*p*-Value
Yield (%)[a]					
Model	233.79	9	25.98	13.24	0.0013
Quadratic	42.61	3	14.20	7.24	0.0150
X_1	19.55	1	19.55	9.97	0.0160
X_2	1.82	1	1.82	0.93	0.3675
X_3	167.06	1	167.06	85.18	<0.0001
X_1^2	9.68	1	9.68	4.93	0.0617
X_2^2	1.05	1	1.05	0.53	0.4886
X_3^2	28.81	1	28.81	14.69	0.0064
X_1X_2	0.23	1	0.23	0.12	0.7398
X_1X_3	2.51	1	2.51	1.28	0.2951
X_2X_3	4.57×10^{-5}	1	4.57×10^{-5}	2.33×10^{-5}	0.9963
Residual	13.73	7	1.96		
Lack of Fit	10.16	3	3.39	3.80	0.1149
Total	247.51	16			
Uronic acid content (mg/g)[b]					
Model	349.25	3	116.42	36.90	<0.0001
Linear	349.25	3	116.42	36.90	<0.0001
X_1	74.42	1	74.42	23.59	0.0003
X_2	3.19	1	3.19	1.01	0.3326
X_3	271.63	1	271.63	86.10	<0.0001
Residual	41.01	13	3.15		
Lack of fit	28.42	9	3.16	1.00	0.5429
Total	390.26	16			
%DPPHsc/g extract (%/g)[c]					
Model	33,077.79	9	3675.31	103.77	<0.0001
Quadratic	9391.66	3	3130.55	88.39	<0.0001
X_1	15,597.25	1	15,597.25	440.38	<0.0001
X_2	1060.12	1	1060.12	29.93	0.0009
X_3	5794.00	1	5794.00	163.59	<0.0001
X_1^2	2949.36	1	2949.36	83.27	<0.0001
X_2^2	1353.08	1	1353.08	38.20	0.0005
X_3^2	4159.56	1	4159.56	117.44	<0.0001
X_1X_2	1004.50	1	1004.50	28.36	0.0011
X_1X_3	227.16	1	227.16	6.41	0.0391
X_2X_3	3.10	1	3.10	0.09	0.7758
Residual	247.93	7	35.42		
Lack of fit	21.40	3	7.13	0.13	0.9399
Total	33,325.72	16			

[a] The coefficient of determination (r^2) of the model was 0.9445.
[b] The coefficient of determination (r^2) of the model was 0.8949.
[c] The coefficient of determination (r^2) of the model was 0.9926.

predicted models in this response was 0.9445 and *p*-value for Lack of Fit was 0.1149. These values would give a relative good fit to the mathematic model in Eq. (5).

3.1.2. Uronic acid content

Linear term of pH (X_3) showed the largest effect ($p < 0.0001$) on uronic acid content followed by extraction temperature time (X_1). Linear term of extraction time was however insignificant ($p > 0.05$) to this contribution. The coefficient of determination (R^2) of the predicted models in this response was 0.8949 and *p*-value for Lack of Fit was 0.5429. These values would give a relative good fit to the mathematic model in Eq. (6).

3.1.3. Antioxidant activity (%DPPHsc/g extract)

In term of antioxidant activity, it can be observed that linear and quadratic terms of extraction temperature (X_1, X_1^2) and pH (X_3, X_3^2) gave the largest effect ($p < 0.0001$) followed by quadratic term of extraction time (X_2^2), linear term of extraction time (X_2), interaction term of X_1X_2 and X_1X_3. However, interaction term of X_2X_3 was not significant ($p > 0.05$). The coefficient of determination (R^2) of the predicted models in this response was 0.9926 and *p*-value for Lack of Fit was 0.9399, which suggesting an excellent fit to the mathematical model (Eq. (7)). The predicted models seemed can reasonably represent the observed values. Thus, the responses were sufficiently explained by the models.

3.2. Interpretation of response surface model and contour plot

3.2.1. Extraction yield

Three-dimensional (3D) plot and contour plot for extraction yield as a function of extraction temperature and pH is given in Fig. 1. Result given in Table 3 shows that incubation time was not significantly ($p > 0.05$) contributed to this response. Therefore, 3D plot was only shown at zero level of extraction time (3 h). It can be observed that the pH demonstrated an exponential increase on the response when pH increased (Fig. 1) whereas extraction temperature has a slight negative impact (i.e. yield decreased with the increment of temperature). Result also shows that at the region where high pH (pH > 3.5) irrespective of temperature would give a high yield of EPP (≥17.6%). As pH decreased, EPP yield decreased to 14.7% at extraction temperature higher than 55.5 °C. Lower temperature would give higher EPP yield ranging from 15.4 to 16.1%. According to Renard, Crépeau, and Thibault (1995) and Wang, Pagán, and Shi (2002), this occurrence might due to the induced cell wall disruption and solubilisation of cell wall materials at higher pH condition.

3.2.2. Uronic acid content

On the other hand, increased the extraction temperature and lowered the pH condition would increased the uronic acid content of the EPP. This is contrary to the result obtained in extraction

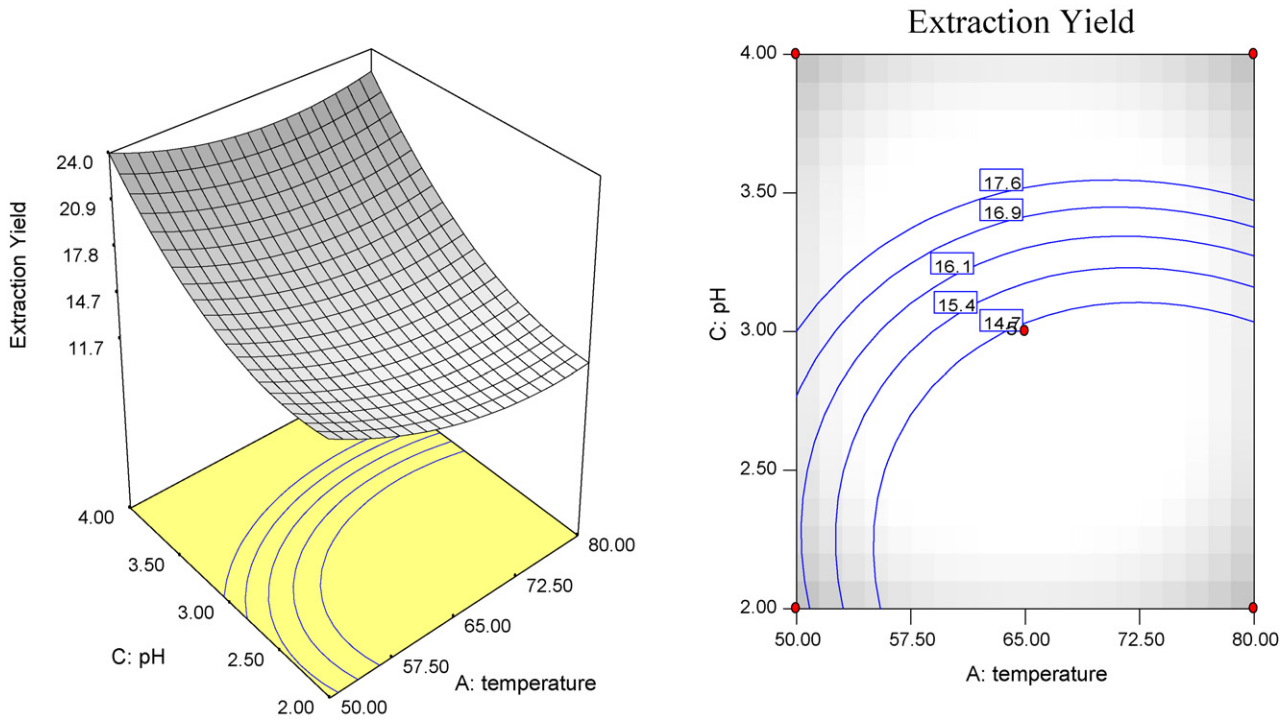

Fig. 1. Three-dimensional plot contour plot for extraction yield as a function of temperature and pH at incubation period of 3 h.

yield. This occurrence suggested that the total yield of EPP might not contributed only by pectic-polysaccharide. Three-dimensional response surface for uronic acid content of EPP as a function of extraction temperature and pH is given in Fig. 2. Again, incubation time was not significantly ($p > 0.05$) contributed to this response (Table 3) and therefore the response surface was only shown at zero level of extraction time (3 h). Fig. 2 shows that high uronic acid content (≥16.2 mg/g) could be achieved at region of pH 3.1 and temperature of 80.0 °C or pH 2.0 and temperature of 50.0 °C. As pH increased with decrement of temperature, this response decreased ranging from 11.3 to 15.0 mg/g. This could be explained that lower pH and high temperature favours the extraction of pectic-polysaccharide through disruption of the ester linkages and hydrogen bonds between EPP and cell wall and increased the diffusion rate of EPP (Renard et al., 1995; Wang et al., 2002; Cho & Hwang, 2000; Masmoudi et al., 2008).

3.2.3. Antioxidant activity (%DPPHs/g extract)

The 3D response surfaces and contour plots for %DPPHsc/g extract are given in Fig. 3a–c. It was apparent that EPP possessed

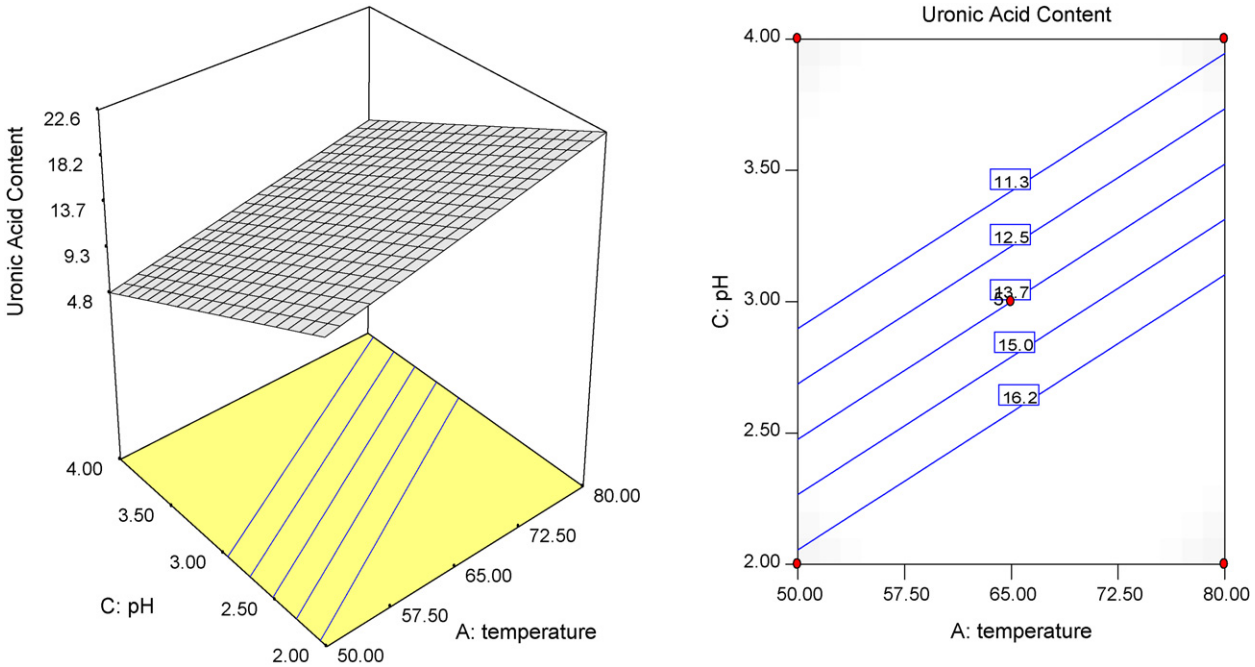

Fig. 2. Three-dimensional plot and contour plot for uronic acid content as a function of temperature and pH at incubation period of 3 h.

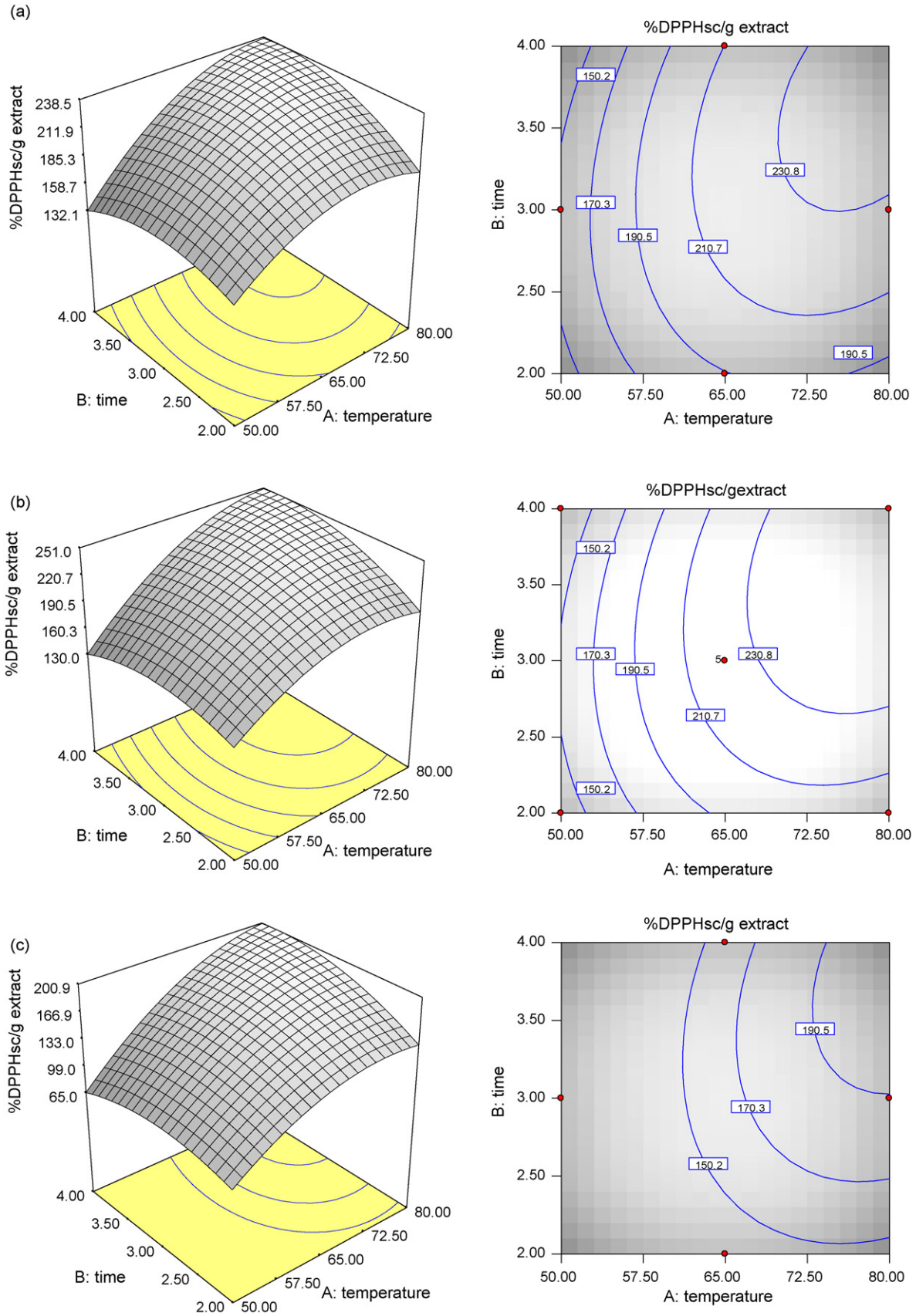

Fig. 3. Three-dimensional plots and contour plots for %DPPHsc/g extract as a function of temperature and time at different pH: (a) pH 2; (b) pH 3; (c) pH 4.

Table 4
Predicted and experimental values at optimum conditions.

Run	Process variables			Predicted values			Experimental values[a]		
	Incubator temperature (°C)	Extraction time (h)	pH	Yield (%)	Uronic acid content (mg/g)	%DPPHsc/g extract	Yield (%)	Uronic acid content (mg/g)	%DPPHsc/g extract
1	80.0	3.93	2.45	12.1	20.6	251.7	12.0	21.1	252.3
2	67.7	3.67	2.00	12.1	20.5	225.1	12.4	20.2	227.2

[a] Mean of triplicate determination.

antioxidant activity by scavenging DPPH free radicals. It could also be observed that the increment of extraction temperature or decrement of pH value would increase the antioxidant activity. This is consistent with the result obtained from uronic acid content (Section 3.2.2). Extract from pH 3.0 or lower seems to obtain a higher antioxidant activity compared to extraction condition of pH more than 3.0. At pH 2.0 (Fig. 3a) and pH 3.0 (Fig. 3b), %DPPHsc/g extract value could reached more than 230.8 at regions of temperature ≥72.5 °C, extraction time ≥3 h and temperature ≥68.8 °C, extraction time ≥2.75 h, respectively. Lowering the extraction temperature resulted in lower antioxidant activity of EPP ranging from 150.2 to 210.7. On the other hand, maximum %DPPHsc/g extract value of ~190.5 could only be achieved at pH 4.0. This might due to higher pH during extraction favours the occurrence of oxidation. Therefore, it could be anticipated that the search for plant-derived biomaterials from this study could generate a natural value-added products (i.e. antioxidant fibre) from under-utilized bulk agro-waste and used as medicinal agent to against chronic health problems such as cancers, inflammation, aging and atherosclerosis caused by reactive free radicals that produced from oxidation (Fridovich, 1978; Kinsella, Frankel, German, & Kanner, 1993).

3.3. Verification of predictive models

Based on the above findings, an optimization study was performed to evaluate the optimal operating conditions for the extraction with high yield of EPP, uronic acid content and antioxidant activity. Table 4 shows two optimum conditions based on combination of all responses. These optimal conditions were (1) extraction temperature of 80.0 °C, extraction time of 3.93 h and pH of 2.45; (2) extraction temperature of 67.7 °C, extraction time of 3.67 h and pH of 2. These optimum conditions yielded EPP of ~12.1%, uronic acid content of ~20.6 mg/g and %DPPHsc/g extract of ~251.7–225.1%. Only small deviations were found between the actual values and predicted values. Thus, the model can be used to optimize the process of EPP extraction from mangosteen rind.

4. Conclusion

RSM was used to determine the optimum process parameters that gave a high extraction yield, uronic acid content and antioxidant activity. According to ANOVA, the effects of extraction temperature, extraction time and pH were significant. Quadratic models fitted to responses of extraction yield and antioxidant activity whereas linear model was fitted to uronic acid content for predicting the responses. Two optimal conditions were determined: (1) extraction temperature of 80.0 °C, extraction time of 3.93 h and pH of 2.45; and (2) extraction temperature of 67.7 °C, extraction time of 3.67 h and pH of 2. Both conditions yielded EPP of ~12.1% and uronic acid content of ~20.6 mg/g. However, conditions (1) and (2) yielded different %DPPHsc/g extract values of ~251.7 and 225.1%, respectively.

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